APPENDIX B–Version with markings to show changes made

1. (Amended) A method of detecting the presence or absence of at least one mismatch between a nucleic acid probe and a nucleic acid target, wherein said probe and target have sequences which differ by not more than five mismatches, said probe comprising a known sequence and a sugar and base free photoactivatable cross-linking agent, which when said probe sequence is hybridized to said target sequence, upon photoactivation forms a covalent bond between said probe sequence and said target sequence, said method comprising:

combining, in a hybridizing medium, a nucleic acid sample comprising said target and said probe under mild stringency hybridizing conditions for a time sufficient for said target and said probe to hybridize;

irradiating said hybridizing medium to form cross-links between said probe and target sequence to which said probe is hybridized to from cross-linked double-stranded nucleic acid;

separating nucleic acid in said hybridizing medium by denaturing electrophoresis and comparing the migratory rate of said cross-linked double-stranded nucleic acid to a known mismatched or matched cross-linked double-stranded nucleic acid standard, whereby the presence or absence of said at least one mismatch is determined.

2. A method according to Claim 1, wherein said probe is labeled with a detectable label.

3. A method according to Claim 1, wherein said sample is prepared using the polymerase chain reaction and said sample nucleic acid is labeled with a detectable label.

4. A method according to Claim 1, wherein said electrophoresis is polyacrylamide gel electrophoresis.

5. (Amended) A method of detecting the presence or absence of at least one mismatch between a nucleic acid probe and a nucleic acid target, wherein said probe and target have sequences which differ by not more than five nucleotide base pair mismatches, said target sequence comprises a nucleic acid molecule of from about 25 to 300 nt and said probe comprising a known polynucleotide sequence of from 15 to 50 nt and a sugar and base free photoactivatable cross-linking agent, which when said probe sequence is hybridized to said target sequence, upon photoactivation forms a covalent bond between said probe sequence and said target sequence, said method comprising:

combining, in a hybridizing medium, a nucleic acid sample comprising said target and said probe under mild stringency hybridizing conditions for a time sufficient for said target and said probe to hybridize;

irradiating at a wavelength in the range of about 300-400 nm said hybridizing medium to form cross-links between said probe and target sequence to which said probe is hybridized to cross-linked double-stranded nucleic acid;

separating nucleic acid in said hybridizing medium by denaturing electrophoresis and comparing the migratory rate of said cross-linked double-stranded nucleic acid to a known mismatched or matched cross-linked double-stranded nucleic acid standard, whereby the presence or absence of said at least one mismatch is determined.

6. A method according to Claim 5, wherein said sample is prepared by restriction enzyme digestion of genomic DNA.

7. A method according to Claim 5, wherein said sample is prepared using the polymerase chain reaction and said sample nucleic acid is labeled with a detectable label.

8. A method according to Claim 5, wherein said probe is labeled with a detectable label.

9. A method according to Claim 5, wherein said electrophoresis is polyacrylamide gel electrophoresis.

10. (Amended) A method of detecting the presence or absence of at least one mismatch between a nucleic acid probe and a nucleic acid target, wherein said probe and target have sequences which differ by not more than five mismatches, said target sequence comprising a nucleic acid molecule of from about 25 to 300 nt and wherein said probe comprises a known polynucleotide sequence of from 15 to 50 nt and a sugar and base free photoactivatable cross-linking agent, which when said probe sequence is hybridized to said target sequence, upon photoactivation forms a covalent bond between said probe sequence and said target sequence, said method comprising:

combining, in a hybridizing medium, a nucleic acid sample comprising said target and said probe under mild stringency hybridizing conditions equivalent to a temperature in the range of 25 -70°C and with 0.1 -1.5 M sodium for a time sufficient for said target and said probe to hybridize;

irradiating at a wavelength in the range of about 300-400 nm said hybridizing medium to form cross-links between said probe and target sequence to which said probe is hybridized to from cross-linked double-stranded nucleic acid;

separating nucleic acid in said hybridizing medium by denaturing gel electrophoresis and comparing the migratory rate of said cross-linked double-stranded nucleic acid to a known mismatched or matched cross-linked double-stranded nucleic acid standard, whereby the presence or absence of said at least one mismatch is determined.

11. A method according to Claim 10, wherein said cross-linking agent comprises a coumarinyl group.